Immunology 1987 **60** 239–245

Influence of reserpine on *in vivo* localization of injected lymph node cells in the mouse

A. BELLAVIA* & H. S. MICKLEM *Department of Zoology, University of Edinburgh, Edinburgh*

Accepted for publication 17 October 1986

SUMMARY

The effects of reserpine, and other agents that affect the storage and availability of 5-hydroxytryptamine (5HT), on the localization of injected ^{51}Cr-labelled syngeneic lymph node cells have been investigated. A high dose (5 mg/kg) of reserpine to the recipients reduced localization in the lymph nodes and prevented the usual accumulation of lymphocytes in lymph nodes draining the site of an antigen (sheep erythrocytes: SE) injection. These effects were partially reversible by the monoamine oxidase inibitor nialamide. This dose of reserpine produced deep sedation throughout the period of the experiment. Lower doses, up to 2·5 mg/kg, produced little sedation and had no effect on the localization of lymphocytes. Other workers had previously reported reduced localization of cells in delayed-type hypersensitivity (DTH) lesions after treatment of the recipients with 5 mg/kg reserpine, and had interpreted this in terms of a role of 5HT in promoting vascular permeability and egress of blood cells. The effect of lower doses of reserpine was not reported. We suggest that the effects on cell localization in both sets of experiments may have been secondary to the general state of sedation and not attributable to a direct local influence of 5HT. Other effects of reserpine included prolonged retention of lymphocytes in lungs and blood, and a reduction of cellularity and DNA synthesis in the thymus, spleen and lymph nodes.

INTRODUCTION

The drug reserpine has several pharmacological effects, including depletion of storage depots of 5-hydroxytryptamine (5HT) in blood platelets and mast cells (Shore, 1962; Jansson, 1970; Stitzel, 1977), depletion of peripheral stores of catecholamines (Burn & Rand, 1958; Kuntzman *et al.*, 1962) and stimulation of monoamine oxidase (MAO) activity (Izumi *et al.*, 1969). It has been reported to interfere with the elicitation of delayed-type hypersensitivity (DTH) in rats (Draskoci & Jankovic, 1964), guinea-pigs (Polak & Turk, 1969) and mice (Gershon, Askenase & Gershon, 1975).

It has been suggested that the depression of dermal DTH by reserpine may be related to depletion of 5HT in the skin, resulting in lack of permeability of venules and hence failure of mononuclear cells to emigrate and set up the lesion (Gershon *et al.*, 1975). This view has been reinforced by reports of direct effects of 5HT on venule endothelium (Schwartz, Askenase & Gershon, 1977). In addition, mast cell degranulation and increased permeability (reversible by reserpine and the 5HT-antagonist cyproheptadine) of venule endothelium in DTH lesions have been described (Askenase *et al.*, 1980). However, the belief that 5HT is important in the development of DTH lesions has been challenged by the demonstration that mast cell-deficient mice can give normal DTH responses (Thomas & Schrader, 1983; Galli & Hammel, 1984). Irrespective of their interpretation, the data of Gershon *et al.* (1975) showed that high doses of reserpine could inhibit DTH, and that this inhibition was partly reversible by the MAO-inhibiting drug nialamide.

Parallels have been drawn between the lymph nodes and antigen-induced DTH lesions (Rannie, Smith & Ford, 1977). Both exist as accumulations of cells that migrate into the tissue from the bloodstream via venular endothelium, and represent sites of contact between lymphocytes and antigen. They differ in that lymph nodes, although their size and cellular composition vary in response to antigenic stimulation, are permanent structures.

One of the early events after local stimulation with a variety of antigens is an increase in the rate of lymphocyte migration to the regional lymph nodes (Zatz & Lance, 1971; Inchley *et al.*, 1976) due, at least in part, to increased blood flow to the node

*Present address and correspondence: Dr A. Bellavia, Istituto di Patologia Generale, Universita di Palermo, 90134 Palermo, Italy.

Abbreviations: DTH, delayed-type hypersensitivity; 6HD, 6-hydroxydopamine; HEV, high endothelial venule; 5HT, 5-hydroxytryptamine (serotonin); IUdR: 5-iodo-2′-deoxyuridine; LN, lymph node; MAO, monoamine oxidase; PBS, phosphate-buffered saline (pH 7·2); SE, sheep erythrocytes.

(Hay & Hobbs, 1977). One result is a gain in weight and cellularity of the lymph node over a period of several days. The factors that contribute to these events, by influencing the behaviour of the local microvasculature, are largely unknown. We now show that reserpine, 5HT antagonists and nialamide can all influence lymphocyte traffic to the lymph nodes, and that their effect is most marked after antigenic stimulation. The results are consistent with the idea that 5HT is among the factors that influence the microvascular contribution to lymphocyte traffic in the mouse. However, it seems probable that reserpine and 5HT antagonists, in the very high doses required to produce an effect, have other ill-understood influences on lymphocyte localization.

MATERIALS AND METHODS

Mice

Highly inbred CBA/Ca and CBA/H-T6 mice were bred and maintained in this laboratory. Males and females were used between 3 months and 6 months of age. Within each experiment, mice were matched for strain, sex and age (within 2 weeks).

Drugs and other materials

Reserpine (Serpasil solution for parenteral injection: Ciba, Summit, NJ) was received as gifts from Dr P. W. Askenase (Dept. of Medicine, Yale University) and Dr D. M. V. Parrott (Dept. of Bacteriology, Glasgow University). Mice received a single intraperitoneal (i.p.) dose of up to 5 mg/kg. Controls received an appropriate volume of the solvent mixture used in Serpasil. For some experiments, reserpine powder (Sigma, Poole, Dorset) was dissolved in 20 mg/ml aqueous ascorbic acid. Cyproheptadine (Periactin) was obtained as a gift from Merck, Sharp & Dohm Ltd, London. It was dissolved in phosphate-buffered saline, pH 7·2 (PBS), and injected i.p. in a single dose of up to 90 mg/kg. Methysergide (Deseril: Sandoz, Basel, Switzerland) was obtained in tablet form, dissolved by crushing in PBS and injected i.p. as a single dose of 25 mg/kg. Nialamide, 5-hydroxytryptamine creatinine sulphate complex (5HT) and 6-hydroxydopamine HBr (6HD) were obtained from Sigma. Nialamide was dissolved in a few drops of concentrated HCl and the pH adjusted to 5·0 by the addition of 1 N NaOH. Distilled water was added to bring the concentration to 10 mg/ml. It was injected i.p. in multiple doses each of 20 mg/kg at 4–6 hr intervals. 5HT was dissolved in PBS and single doses of up to 50 μg were injected into each front footpad in a volume of 0·05 ml. 6HD was dissolved in 1 mg/ml aqueous ascorbic acid and injected i.p. or subcutaneously (s.c.) in a single dose of 250 mg/kg.

All solutions were prepared immediately before use. Control mice received equal volumes of the appropriate diluent.

Sodium ^{51}chromate (^{51}Cr) and 5-^{125}iodo-2′-deoxyuridine (IUdR) were obtained from Amersham International, Amersham, Bucks. Sheep red blood cells (SE) in Alsever's solution were obtained from Tissue Culture Services, Slough, Berks.

Immunization

SE were washed three times in PBS; 5×10^7 in a volume of 0·05 ml were injected s.c. into the left front footpad. Controls received the same volume of PBS.

Lymphocyte localization assay

Lymph node (LN) cell suspensions were prepared and radiolabelled with ^{51}Cr as previously described (Bellavia & Micklem, 1977). Radiolabelled cells, $5–10 \times 10^6$, were injected intravenously (i.v.) into each syngeneic recipient. This step was performed 21–24 hr after immunization with SE. At various times thereafter, recipients were bled and then killed by cervical dislocation. The following tissues were removed and counted in a Nuclear Enterprises Model 8311 automatic gamma counter: individual brachial, axillary and inguinal lymph nodes, spleen, liver, lungs and a measured sample of blood. In many experiments the mesenteric lymph node chain, Peyer's patches (pooled) and small intestine were also counted. Samples of the injected cells were counted and the results were expressed as a percentage of the injected radioactivity (normally 30,000–50,000 c.p.m.) in each organ or in the total blood volume. The latter was assumed to be 1·5 ml.

Cell proliferation assay

The proliferative activity in lymph nodes, spleen and thymus was assessed by measuring the *in vivo* incorporation of ^{125}IUdR into DNA, as described by Pritchard & Micklem (1972). Organs were counted for 10 min in the gamma counter, results being expressed as the geometric mean percentage of the radioactivity (normally 10^6 c.p.m.) injected i.p. initially.

Statistical analysis and presentation of results

All groups consisted of three to five mice. In some experiments, results are presented as the mean $\pm$SEM. In others, for the sake of brevity, they are presented as ratios between the experimental and control groups. A ratio <1 indicates that less ^{51}Cr was found in the experimental than the control mice, and vice versa. The significance of the differences obtained between experimental and control groups was estimated by Student's *t*-test.

RESULTS

Influence of reserpine on cell localization

The kinetics of localization of radiolabelled lymph node cells injected 24 hr after reserpine (5 mg/kg) are shown in Fig. 1a–c. In this experiment SE were injected s.c. into the left front footpad immediately after the reserpine. The effects of reserpine were as follows. (i) Antigen-related cell localization in the regional lymph node was suppressed, and localization in contralateral and mesenteric lymph nodes was also reduced. (ii) Splenic localization was reduced, so that 30 min after cell injection three times less radioactivity was present in the reserpine-treated mice; the difference decreased with time, but was still evident at 6 hr. A comparable reduction was seen in 13/13 further experiments in which 3-hr localization was studied. (iii) Peyer's patch localization was increased 2–6 hr after cell injection in this experiment. However, taking eight further experiments into account, the effects on Peyer's patch localization were quite inconsistent. Localization in the small intestine, excluding the Peyer's patches, showed a slight increase in 7/8 experiments (data not shown). (iv) There was a 1–2 hr delay in the disappearance of the injected cells from the blood and lungs. Blood localization was also elevated at 3 hr in 14/14 further experiments. (v) No differences in liver localization were seen here, or in 12 further experiments (data not shown). An average of 10% of injected counts were recovered in the liver.

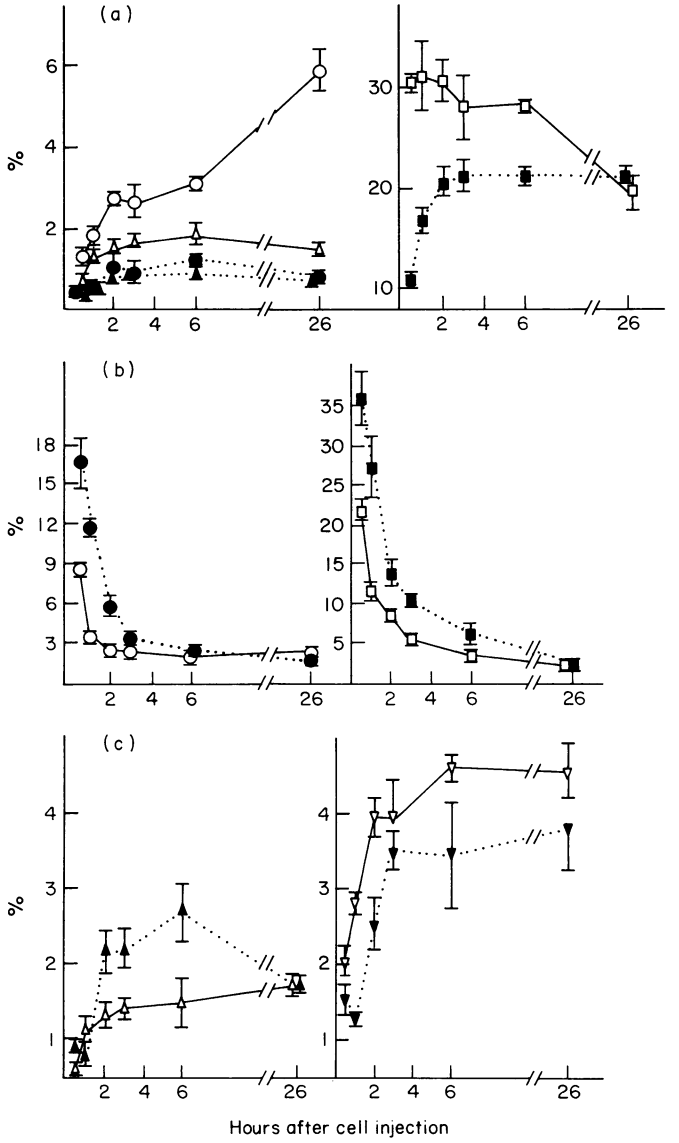

Figure 1. Localization of radiolabelled lymph node cells as a function of time in reserpine-treated (solid symbols) and control (open symbols) mice. Reserpine was injected i.p. and SE s.c. (into the left front footpad) 24 hr before the cell inoculum. (a) Draining brachial (●,○) and contralateral brachial (▲,△) lymph nodes and spleen (■,□); (b) blood (●,○) and lungs (■,□); (c) Peyer's patches (▲,△) and mesenteric lymph nodes (▼,▽).

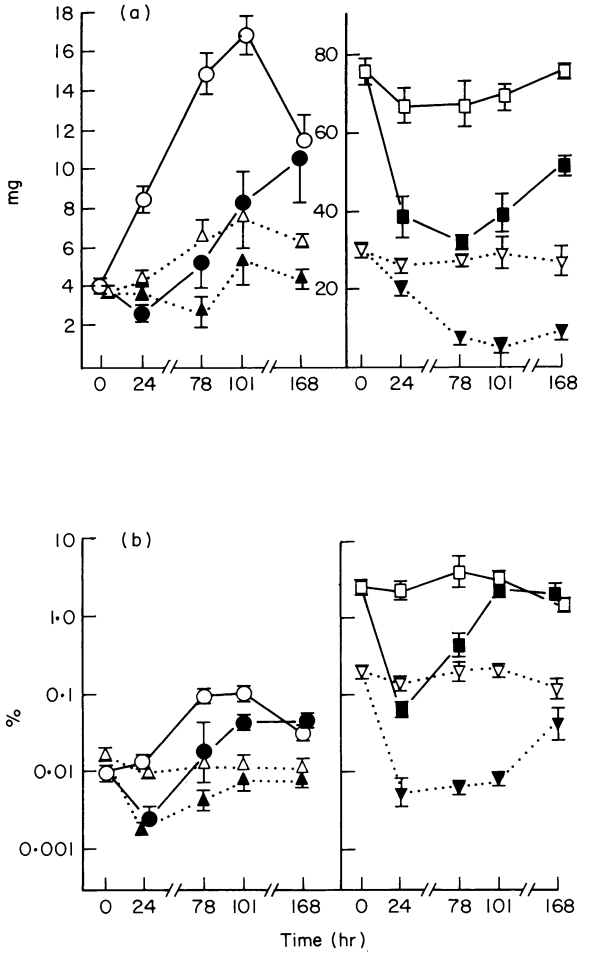

Figure 2. Weight (a) and ^{125}I-IUdR incorporation (b) of lymphoid organs as a function of time after i.p. injection of reserpine (solid symbols) or vehicle (open symbols). SE were injected s.c. into the left front footpad immediately after reserpine (or vehicle) treatment. (●,○) Draining brachial lymph nodes; (▲,△) contralateral brachial lymph nodes; (■,□) spleen; (▼,▽) thymus.

Influence of reserpine on weight and proliferative activity of lymphoid organs

It was noted in the previous experiments that the regional lymph nodes and spleen of reserpine-treated mice were abnormally small. We therefore followed weight changes and IUdR incorporation in lymph nodes, spleen and thymus in a further series of mice (Fig. 2a–b). Splenic weight was reduced by almost 50% within 24 hr and had only partly recovered after 1 week. IUdR incorporation fell by more than 20-fold, but recovered more rapidly, reaching the control level by 101 hr. In the thymus a broadly similar pattern was seen, although the fall in weight and the recovery in IUdR uptake both occurred more slowly than in the spleen. Lymph nodes, too, were markedly affected by reserpine, weight and IUdR incorporation being depressed and the proliferative response to antigenic stimulation delayed 2–3 days.

Lack of effect of lower doses (< 5 mg/kg) of reserpine

The 5 mg/kg dose of reserpine used above was chosen on the basis of earlier reports (Gershon *et al.*, 1975). However, this had a severely prostrating effect on the mice, which were inactive and cold to the touch and, in the absence of extra external warmth, died within 72 hr. Doses between 2·5 mg/kg and 0·02 mg/kg had no significant effects on cell localization (Table 1). The dose of 5 mg/kg was adhered to in the following experiments.

Time–course of the effect of reserpine on cell localization

Reserpine was injected into mice 6, 16, 20 or 28 hr before the injection of radiolabelled cells. The effects of the drug on cell

Table 1. Effect of reserpine on the 3-hr localization of radiolabelled syngeneic lymph node cells

	Radioactivity recovered (ratio experimental:control)				
	Lymph nodes:				
Dose of reserpine (mg/kg)	Left brachial	Right brachial	Right inguinal	Spleen	Blood
5·0	0·15*	0·30*	0·14*	0·93	2·87*
2·5	0·64	1·14	1·02	0·73	0·97
1·0	0·98	1·24	0·98	0·74	1·03
0·5	1·36	1·24	1·35	0·75	1·22
0·1	0·88	0·72	1·11	0·50	0·67
0·02	0·86	0·85	1·14	1·04	0·70

Experimental mice received the stated dose of reserpine i.p. 18 hr before i.v. lymph node cells. Controls received vehicle only. All mice received SE in the left front footpad 24 hr before the cell inoculum.

*$P < 0.001$ for comparison between experimental and control groups. All other differences were non-significant ($P > 0.05$).

Table 2. Influence of reserpine (5 mg/kg) injected at different times on the 3-hr localization of radiolabelled syngeneic lymph node cells

	Radioactivity recovered (ratio experimental:control)				
Time of reserpine injection	Lymph nodes: Left brachial	Right brachial	Spleen	Peyer's patches	Blood
6 hr	0·20*	0·22**	0·89	0·66*	2·63**
16 hr	0·09**	0·13***	0·57	0·55	ND†
20 hr	0·07**	0·18***	0·70	0·35**	3·74**
28 hr	0·14**	0·37*	0·89	0·71	ND

Reserpine (experimental) or vehicle (control) was injected i.p. at the stated times before i.v. injection of radiolabelled cells. SE were injected into the left front footpad 21 hr before the cell inoculum. For reasons detailed in Table 3, experimental and control mice also received up to five i.p. injections of PBS at 4–6 hr intervals between the administration of reserpine and of radiolabelled cells.

Statistical comparison of experimental and control groups: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

† ND, not determined.

localization were greatest at 16 and 20 hr, and only slightly less at 6 and 28 hr (Table 2). This broad plateau of response is in accord with the general state of the animals, which remained heavily sedated for at least 48 hr after the injection of reserpine.

Partial reversal of the effects of reserpine by nialamide

If the effects of reserpine on cell localization are due, at least in part, to depletion of 5HT, then an inhibitor of MAO might be expected to reverse them; the catabolism of 5HT, after its initial release from storage depots in the platelets and mast cells, would be inhibited. Nialamide did in fact partially reverse the effects of reserpine for at least 20 hr, but not at 28 hr when its effect was, if anything, additive to that of reserpine (Table 3). Nialamide by itself, when used according to the same schedule, produced some minor decrease in the localization of cells in lymph nodes (Table 3).

Influence of cyproheptadine and methysergide on cell localization

Cyproheptadine acts as a competitive antagonist of 5HT, as well as being a potent anti-histaminic agent. As shown in Table 4, its effects on cell localization were similar in kind to those of reserpine. In this experiment, localization was measured 1 hr after cell injection, rather than 3 hr as in the experiments on reserpine detailed in Tables 1–3. This difference may account for the relatively small effect of cyproheptadine measured in lymph nodes and the relatively large effect measured in spleen. Again, the drug doses were large (i.p. LD_{50} for our mice = 50 mg/kg). Doses below 45 mg/kg had no significant influence on cell localization. Methysergide, another 5HT antagonist, did not affect lymph node localization at the dose used (25 mg/kg) and did not act additively with 23 mg/kg cyproheptadine (data not shown). Thus, it is uncertain whether the observed decrease in lymphocyte traffic to lymph nodes should be attributed to the major, anti-5HT/anti-histamine, effects of cyproheptadine, or to some other effect.

Influence of the catecholamine depleting agent 6-hydroxydopamine (6HD) on cell localization

Since reserpine could, in principle, exert its influence on cell localization by depleting stores not only of 5HT, but also (or alternatively) of catecholamines, we tested the effect of 6HD, a catecholamine depleter (Kostrzewa & Jacobowitz, 1974). A single large dose of 6HD produced a small but significant *increase* in localization in the lymph nodes (Table 5). This extended to all of the subcutaneous lymph nodes, those for which data are tabulated being typical. The Peyer's patches were not affected and the spleen not significantly so. These results suggested that catecholamine depletion had an effect on lymphocyte localization opposite to that seen after injection of reserpine.

Influence of 5HT on cell localization

An attempt was made to demonstrate a direct effect of locally administered 5HT on cell localization in lymph nodes. 5HT was injected in doses of 0·1 μg, 1 μg and 10 μg into both front footpads 5 min before the injection of labelled cells. Twenty-one hours previously, SE had been injected into the left front footpad. Two separate experiments were performed. Although ^{51}Cr counts were more variable in the brachial lymph nodes of both 5HT-injected and saline-injected control mice than in more distant lymph nodes or other organs, no consistent effect of 5HT was noted at any site or at any of the doses used (data not shown).

DISCUSSION

Several factors influence the numbers of lymphocytes localizing in tissues and organs from the bloodstream: (i) the identity of the cells; (ii) the concentration of cells in the blood; (iii) the blood flow to the tissue or organ concerned; (iv) the permeability of the

Table 3. Partial reversal by nialamide of the effect of reserpine on 3-hr lymphocyte localization

Time of reserpine injection	Radioactivity recovered (ratio experimental:control)				
	Lymph nodes:				
	Left brachial	Right brachial	Spleen	Peyer's patches	Blood
6 hr	2·52*	1·77	1·20	1·74*	0·43**
16 hr	5·26**	3·59**	1·88	5·01**	ND†
20 hr	3·76**	1·36	1·56	4·83	0·46***
28 hr	0·54	0·34	0·98	1·63	ND
No reserpine‡	0·47	0·31	0·98	0·91	0·79

Reserpine (5 mg/kg) was injected i.p. at the stated times before i.v. injection of radiolabelled lymph node cells. Nialamide (20 mg/kg/dose) (experimental) or PBS (control) was injected i.p. at the same time as the reserpine, and then at 4–6 hr intervals until the injection of radiolabelled cells. SE were injected into the left front footpad 21 hr before the cell inoculum. The controls are the same individuals as the experimental mice in Table 2.

Statistical comparison of nialamide- and PBS-injected mice: $*P<0.05$, $**P<0.01$, $***P<0.001$.

† ND, not determined.

‡ These mice received five doses of nialamide or PBS.

Table 4. Influence of cyproheptadine on 1-hr localization of radiolabelled syngeneic lymph node cells

Dose of cyproheptadine	Radioactivity recovered (ratio experimental:control)							
	Lymph nodes:							
	Left brachial	Right brachial	Mesenteric	Spleen	Lungs	Peyer's patches	Small intestine	Blood
90 mg/kg	0·60**	0·59**	0·73	0·29*	2·75*	1·04	1·14	3·23*
45 mg/kg	0·66*	0·58*	0·74	0·65	1·68	0·79	1·13	2·05
23 mg/kg	1·22	1·17	1·01	1·03	1·59	1·24	0·72	1·71

Cyproheptadine (experimental) or PBS (control) was injected i.p. 1 hr before the i.v. injection of radiolabelled cells. SE were injected into the left front footpad 21 hr before the cell inoculum.

Statistical comparison of experimental and control groups: $*P<0.05$, $**P<0.01$.

Table 5. Influence of 6-hydroxydopamine (6HD) on 3-hr localization of radiolabelled syngeneic lymph node cells

Radioactivity recovered (ratio experimental:control)				
Lymph nodes:				
Left brachial	Right brachial	Spleen	Peyer's patches	Blood
1·66*	2·70**	0·83	0·95	1·05

6HD (250 mg/kg) (experimental) or vehicle (control) was injected i.p. 21 hr before the i.v. injection of radiolabelled cells. SE were injected into the left front footpad 24 hr before the cell inoculum.

Statistical comparison of experimental and control groups: $*P<0.05$, $**P<0.01$.

local microvasculature, specifically the high endothelial venules (HEV); (v) the rate of cell egress from the tissue via the efferent lymphatics. Blood flow is itself determined by (a) the cardiac output, which limits the potential flow available, and (b) the constriction/dilation of local blood vessels and the regulation of arterio-venous shunts, which together determine what share of the blood each portion of the body receives. In our experiments, the first factor was held constant by using a single pool of lymph node cells which were injected into all the groups in a given experiment. The fifth factor was assumed to be unimportant in most of the present experiments, since the time between cell injection and death of the recipient (1–3 hr) was much shorter than the reported transit time for lymphocytes through lymph nodes in the mouse (Sprent, 1973). The other factors are potentially important variables, all of which may be influenced by a number of pharmacological and neuropharmacological agents. Gershon *et al.* (1975) emphasized the possible role of

5HT in regulating the blood–tissue passage of cells in dermal cellular hypersensitivity lesions. In particular, the drug reserpine, which depletes the stores of 5HT present in mast cell granules, was found by them to inhibit the localization of blood-borne cells. This effect was partly reversed by the MAO-inhibitor nialamide, which is expected to inhibit the breakdown of 5HT released from the mast cells by reserpine. The catecholamine depletor 6HD did not affect cell localization, so it seemed improbable that reserpine was affecting cell localization through its effect on catecholamine levels. In a subsequent histological study (Askenase *et al.*, 1980) the same group showed that mast cell degranulation did indeed occur in DTH lesions and that the HEV became more permeable through the development of gaps between adjacent endothelial cells.

Our own experiments were partly modelled on those of Gershon *et al.* (1975). They showed that reserpine had effects on lymphocyte localization in antigen-stimulated lymph nodes that were very similar to those previously described in dermal DTH lesions. The 5HT-antagonist cyproheptadine also produced such effects. Thus, our data suggest that similar mechanisms may affect the localization of cells in lymph nodes and DTH lesions. In particular, they suggest that 5HT may be important at some level. However, the dose of reserpine used by Gershon *et al.* (1975) was about 100 times higher than those that have been used in medical and veterinary practice. We noticed its prostrating effect on the recipients, and tested lower doses. Reduction of the reserpine dose by a little as one-half completely abolished the effect on lymphocyte localization. It also led to a marked reduction in the overall clinical effect. Since effects on cell traffic are associated with a high dosage of reserpine and overall prostration of the mice, it seems unsafe to ascribe them to any direct or local influence of 5HT. Rather, they seem likely to be indirect and secondary to the more general impairment of physiological function.

Reserpine at the dose of 5 mg/kg had several other interesting effects. It delayed the disappearance of radioactivity from the lungs of the recipients. Temporary entrapment of injected cells in the capillary bed of the lungs occurs in control mice and has been reported previously (Freitas & de Sousa, 1977; Bellavia, Franklin & Micklem, 1980), so reserpine may delay their subsequent release. The fact that blood levels of radioactivity also declined more slowly after reserpine is consistent with this view, but it is also possible that some injected cells were destroyed in the lungs. Reserpine also had marked effects on the weight and proliferative activity of lymphoid organs, including the thymus and spleen, as well as the lymph nodes. No splenic effect was seen under comparable conditions by Mekori, Weitzman & Galli (1985); the explanation for this discrepancy is unknown. The behaviour of the thymus in reserpine-treated mice was striking. Thymic involution was also noted in rats that received multiple injections of reserpine (Draskoci & Jankovic, 1964). The loss of weight and proliferative activity, and the slow recovery, resemble those seen after exposure to high doses of corticosteroids, and suggest that lymphocytes were destroyed on a considerable scale.

Direct *in vitro* effects of reserpine on lymphocytes have recently been described (Mekori *et al.*, 1985). Treated lymphocytes, after subsequent intravenous infusion, showed less proliferation in recipients' lymph nodes in response to antigenic stimulation. The ability to respond to antigen *in vitro* was also abolished. These observations suggest that some of the *in vivo* effects that we found, particularly on the weight and proliferative activity of lymphoid tissues, were due to direct effects of reserpine on lymphocytes themselves rather than on the microvasculature.

The effects of reserpine, at least in the very high doses that have been customary in experiments on DTH and cell traffic, appear to be complex. The present results with reserpine do not exclude a role for 5HT in lymphocyte traffic. Nor does our failure to demonstrate any effect of injected 5HT: that could have been due to problems of quantity, location or timing. However, there seems to be no good reason to interpret them in terms of any direct, local action of 5HT. Data from experiments with mast cell-deficient mice indicate that DTH lesions can develop in the absence of local 5HT (Thomas & Schrader, 1983; Galli & Hammel, 1984). Thus, the local action of 5HT identified in other experiments (Schwartz *et al.*, 1977; Askenase *et al.*, 1980) may not be of overriding importance in the development of DTH. Although extension of our studies to mast cell-deficient mice would be required to prove the point, the general parallelism of our results with those of Gershon *et al.* (1975) suggests that the same applies to the localization and proliferation of lymphocytes in lymph nodes in response to antigenic stimulation.

ACKNOWLEDGMENT

A. Bellavia was supported by a Fellowship from the Consiglio Nazionale della Ricerche, Rome.

REFERENCES

ASKENASE P.W., BURSZTAJN S., GERSHON M.D. & GERSHON R.K. (1980) T cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J. exp. Med.* **152,** 1358.

BELLAVIA A., FRANKLIN V. & MICKLEM H.S. (1980) Effects of dextran sulphate and heparin on lymphocyte localization: lack of involvement of complement and other plasma proteolytic systems. *Int. J. Immunopharmacol.* **2,** 311.

BELLAVIA A. & MICKLEM H.S. (1977) T lymphocytes and thymocytes with different localization properties separated by nylon wool filtration. *Transplantation*, **23,** 290.

BURN J.H. & RAND M.J. (1958) The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol.* **144,** 314.

DRASKOCI M. & JANKOVIC B.D. (1964) Involution of thymus and suppression of immune responses in rats treated with reserpine. *Nature (Lond.)*, **202,** 408.

FREITAS A.A. & DE SOUSA M.A.B. (1977) Control mechanism of lymphocyte traffic. A study of the action of two sulphated polysaccharides on the distribution of ^{51}Cr- and [^{3}H]adenosine-labeled mouse lymph node cells. *Cell. Immunol.* **31,** 62.

GALLI S.J. & HAMMEL I. (1984) Unequivocal delayed hypersensitivity in mast cell-deficient and beige mice. *Science*, **266,** 710.

GERSHON R.K., ASKENASE P.W. & GERSHON M.D. (1975) Requirement for vasoactive amines for production of delayed-type hypersensitivity skin reactions. *J. exp. Med.* **142,** 732.

HAY J.B. & HOBBS B.B. (1977) The flow of blood to lymph nodes and its relation to lymphocyte traffic and the immune response. *J. exp. Med.* **145,** 31.

INCHLEY C.J., MICKLEM H.S., BARRETT J., HUNTER J. & MINTY C. (1976) Age-related changes in localization of radiolabelled lymphocytes in the lymph nodes of antigen-stimulated mice. *Clin. exp. Immunol.* **26,** 286.

IZUMI F., OKA M., YOSHIDA H. & IMAIZUMI R. (1969) Stimulatory effect

of reserpine on monoamine oxidase in guinea pig heart. *Biochem. Pharmacol.* **18,** 1739.

JANSSON S.-E. (1970) Effect of chlorpromazine, mepyramine, prenylamine and reserpine on 5-hydroxytryptamine content and fine structure of rat peritoneal mast cells incubated *in vitro*. *Acta physiol. Scand.* **78,** 420.

KOSTRZEWA R.M. & JACOBOWITZ D.M. (1974) Pharmacological actions of 6-hydroxydopamine. *Pharmacol. Rev.* **26,** 199.

KUNTZMAN R., COSTA E., GRESSA L. & BRODIE B.B. (1962) Reserpine and guanethidine action on peripheral stores of catecholamines. *Life Sciences*, **1,** 65.

MEKORI Y.A., WEITZMAN G.L. & GALLI S.J. (1985) Reevaluation of reserpine-induced suppression of contact sensitivity. Evidence that reserpine interferes with T lymphocyte function independently of an effect on mast cells. *J. exp. Med.* **162,** 1935.

POLAK L. & TURK J.L. (1969) The effect of antihypertensive agents on the peripheral manifestations of allergic and other inflammatory reactions in the skin. *Internat. Arch. Allergy appl. Immunol.* **36,** 442.

PRITCHARD H. & MICKLEM H.S. (1972) Immune responses in congenitally thymus-less mice. I. Absence of response to oxazolone. *Clin. exp. Immunol.* **10,** 151.

RANNIE G.H., SMITH M.E. & FORD W.L. (1977) Lymphocyte migration into cell-mediated immune lesions is inhibited by trypsin. *Nature (Lond.)*, **267,** 520.

SCHWARTZ A., ASKENASE P.W. & GERSHON R.K. (1977) The effect of locally injected vasoactive amines on the elicitation of delayed type hypersensitivity. *J. Immunol.* **118,** 159.

SHORE P.A. (1962) Release of serotonin and catecholamines by drugs. *Pharmacol. Rev.* **14,** 531.

SPRENT J. (1973) Circulating T and B lymphocytes of the mouse. I. Migrating properties. *Cell. Immunol.* **7,** 10.

STITZEL R.E. (1977) The biological fate of reserpine. *Pharmacol. Rev.* **28,** 179.

THOMAS W.R. & SCHRADER J.W. (1983) Delayed hypersensitivity in mast cell deficient mice. *J. Immunol.* **130,** 2565.

ZATZ M.M. & LANCE E.M. (1971) The distribution of ^{51}Cr-labeled lymphocytes into antigen-stimulated mice. *J. exp. Med.* **134,** 224.